

Sorting by pools

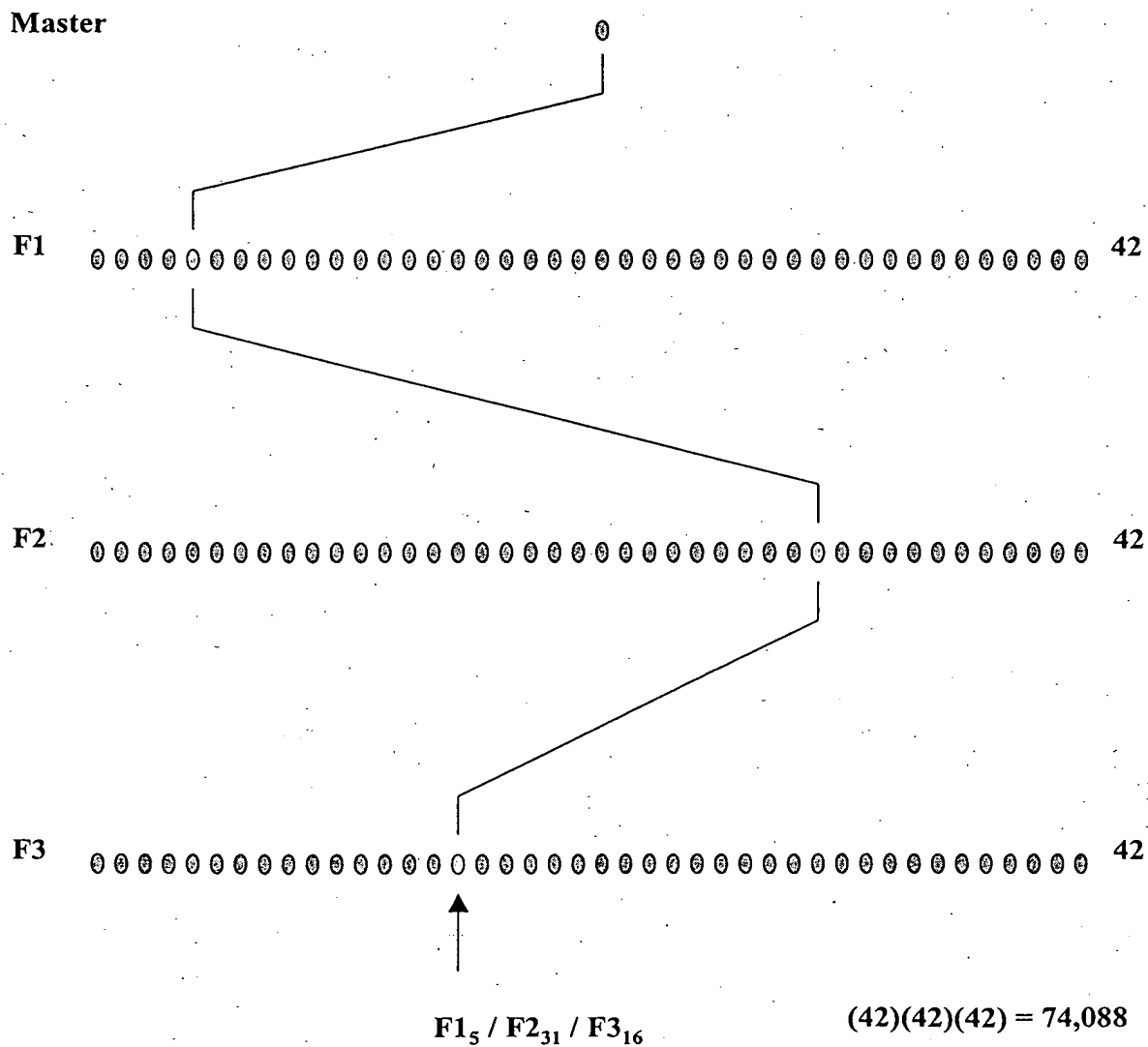


FIGURE 1

Sorting by pools: Decreasing pool diversities

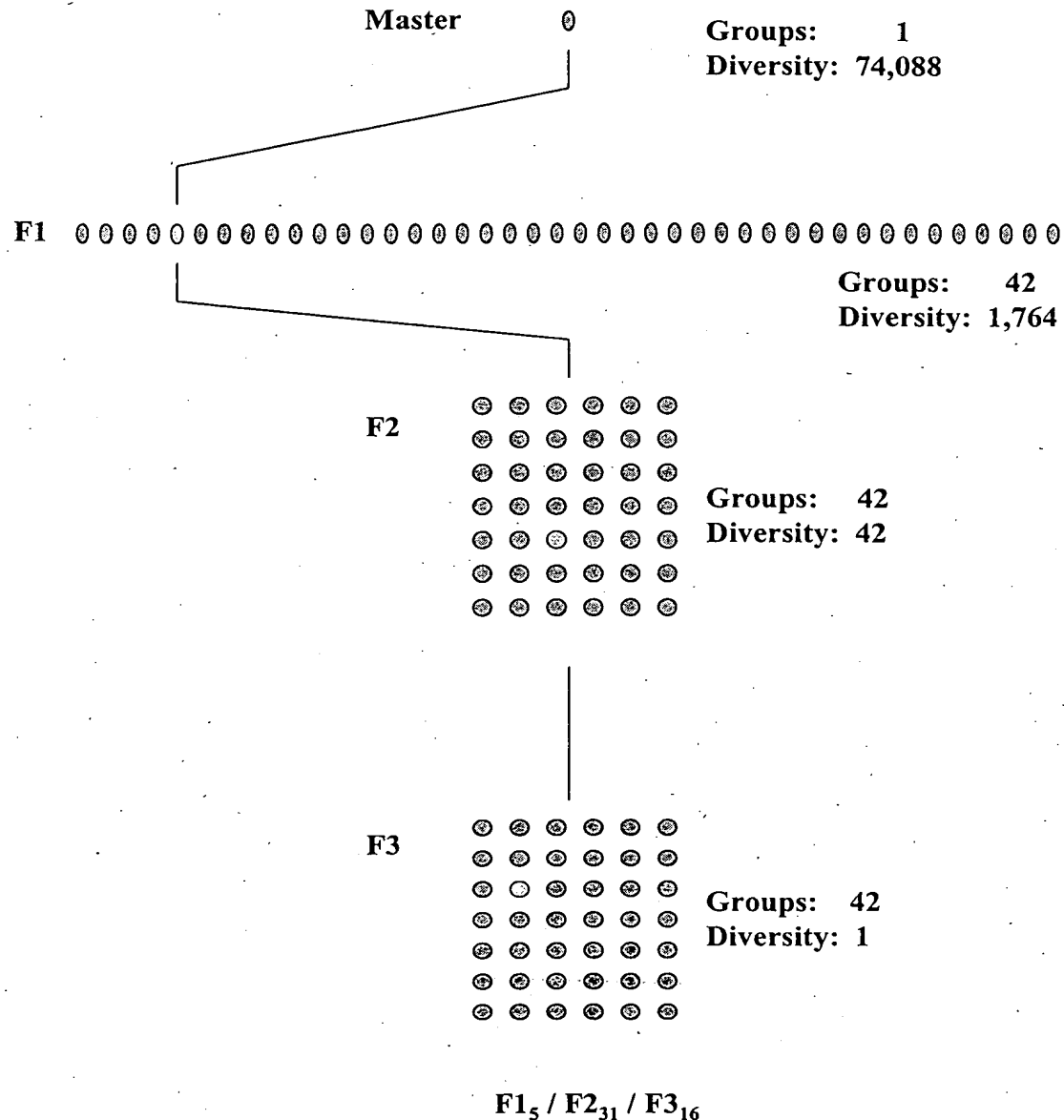


FIGURE 2

Applicant: Ault-Riche *et al.*
DKT. No. 25885-1751

Priority claimed to 60/219,183

For: COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Sorting by pools: Screening large diversity libraries

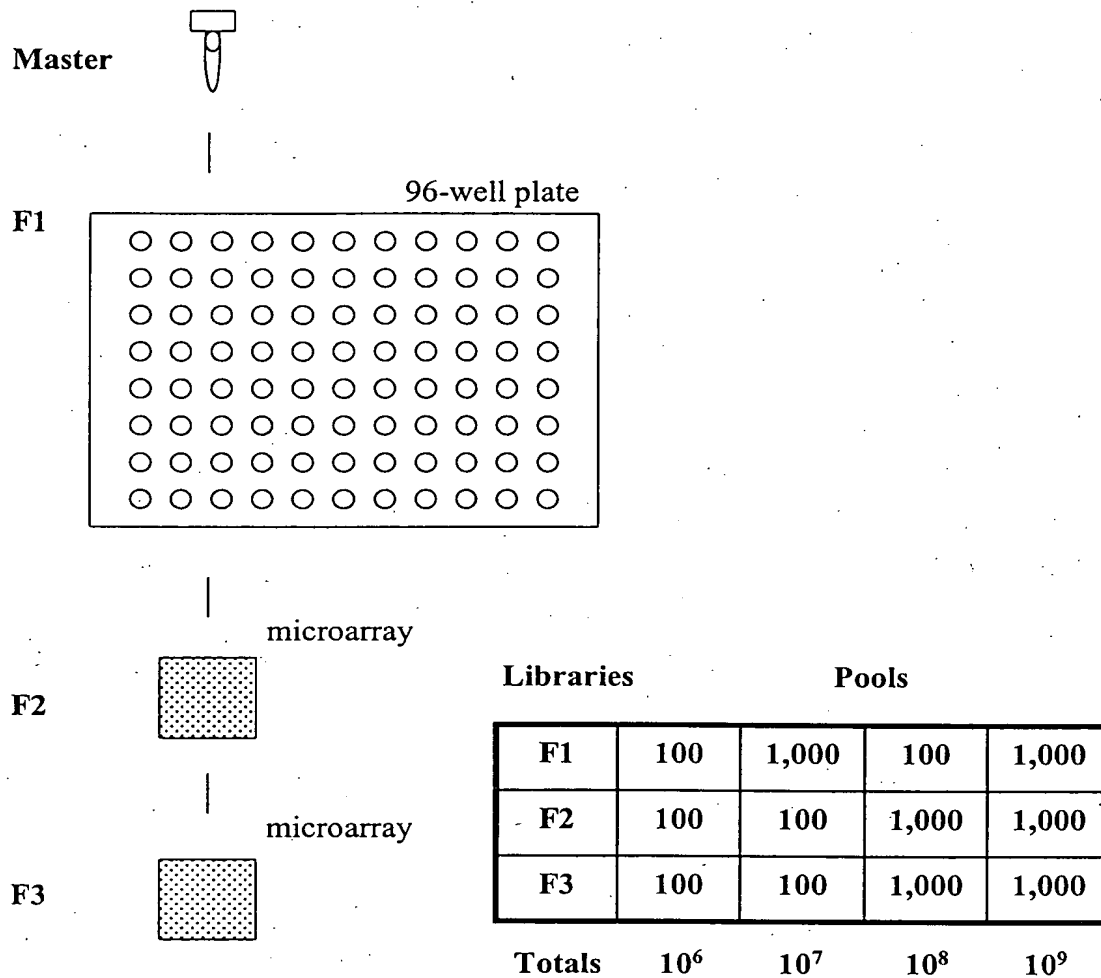


FIGURE 3

Applicant: Ault-Riche *et al.*

DKT. No. 25885-1751

Priority claimed to 60/219,183

For: COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Searching a mutation library

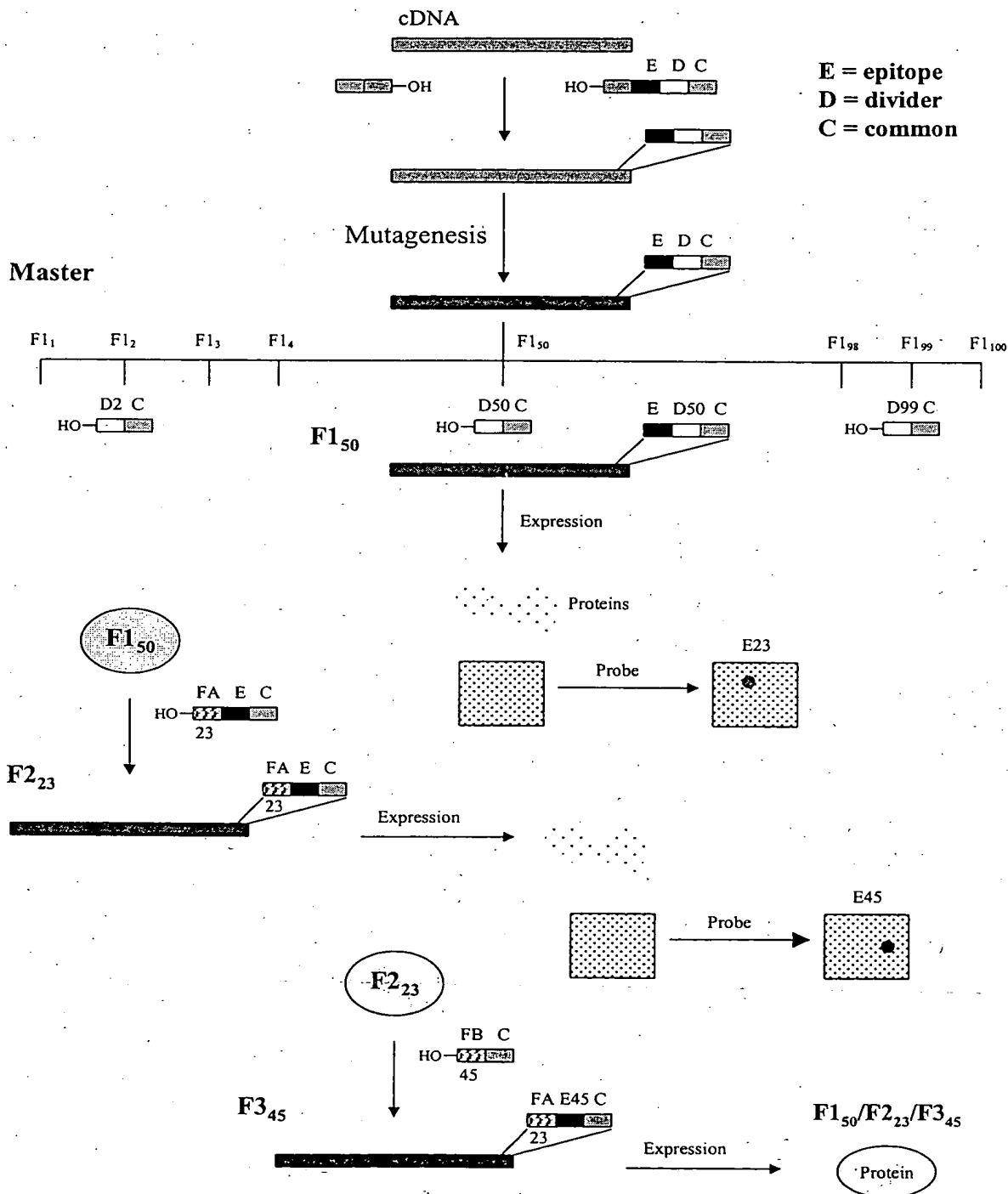
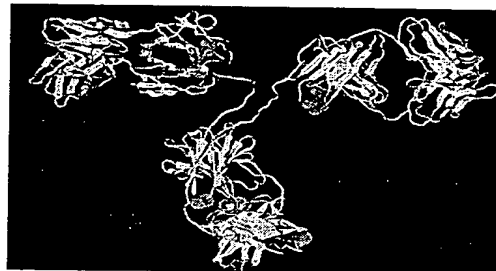
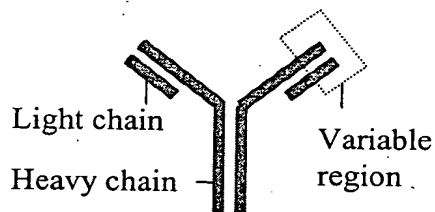


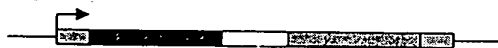
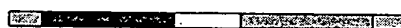
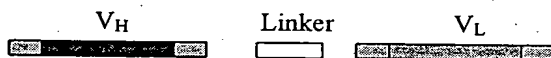
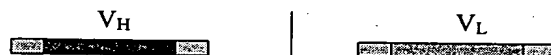
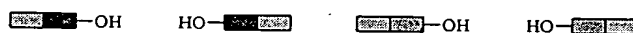
FIGURE 4

Making a recombinant antibody library

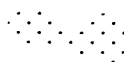
Basic antibody structure



Spleen cells or PBLs



Expression



Antibodies

FIGURE 5

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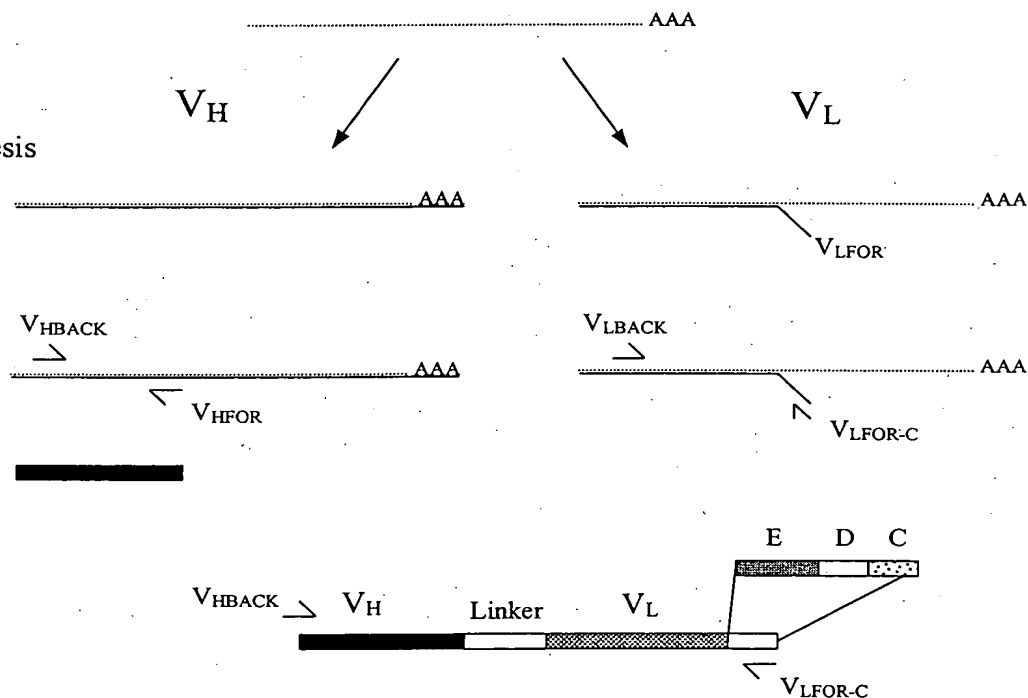
Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs

2. cDNA synthesis

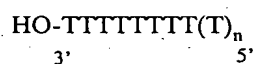
3. amplification

4. assembly

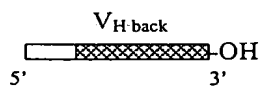


V_H Primers

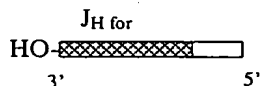
Oligo dT



V_HBACK

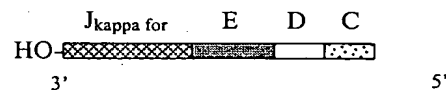


V_HFOR

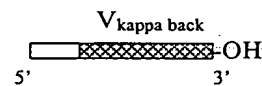


V_L Primers

V_LFOR



V_LBACK



V_LFOR-C

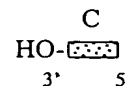


FIGURE 6

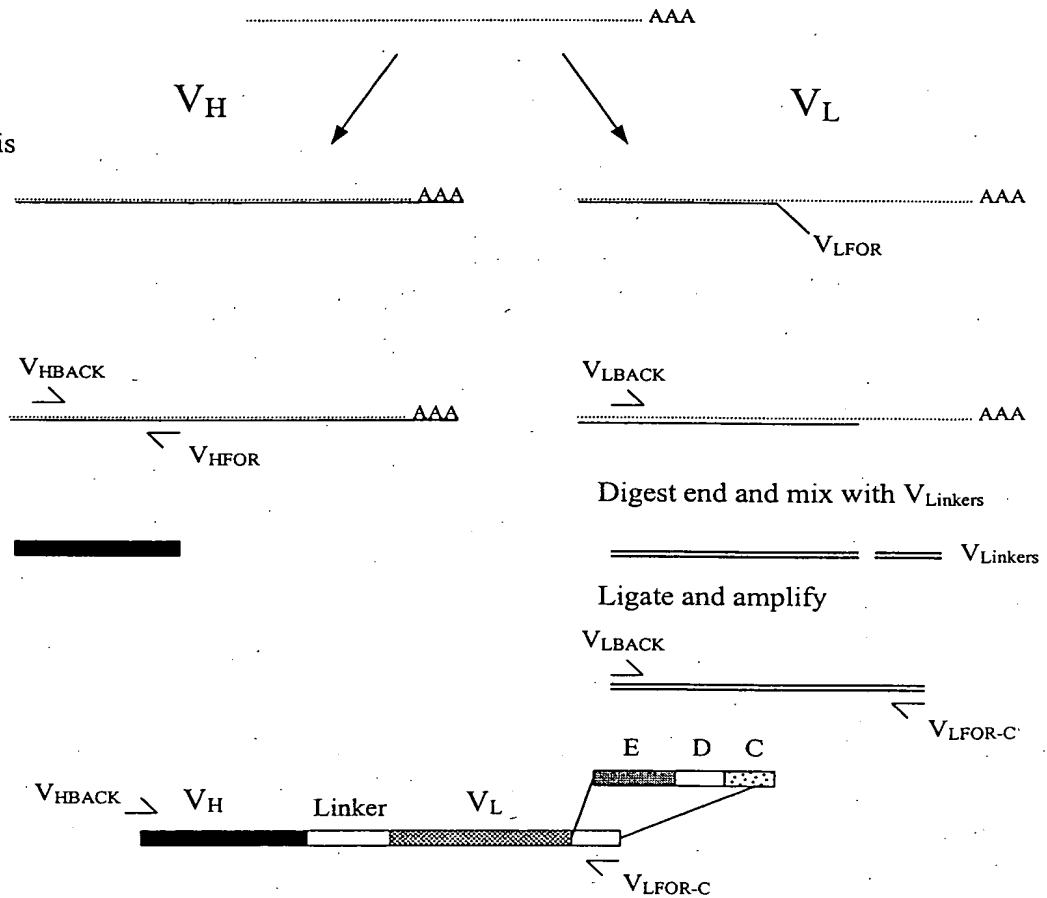
Creating the master antibody library: Linker addition

1. mRNA purification from spleen or PBLs

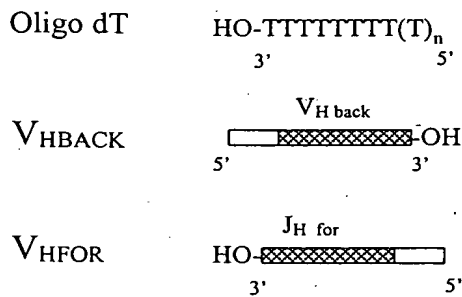
2. cDNA synthesis

3. amplification

4. assembly



V_H Primers



V_L Primers

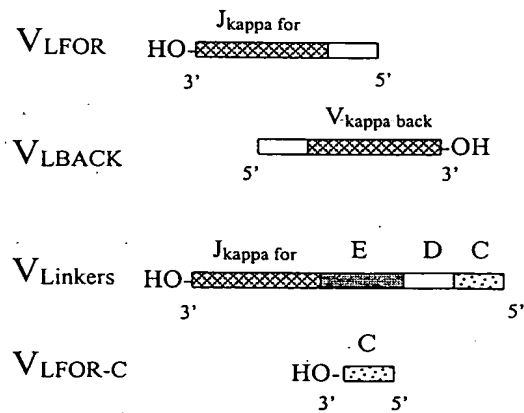


FIGURE 7

Searching a recombinant antibody library

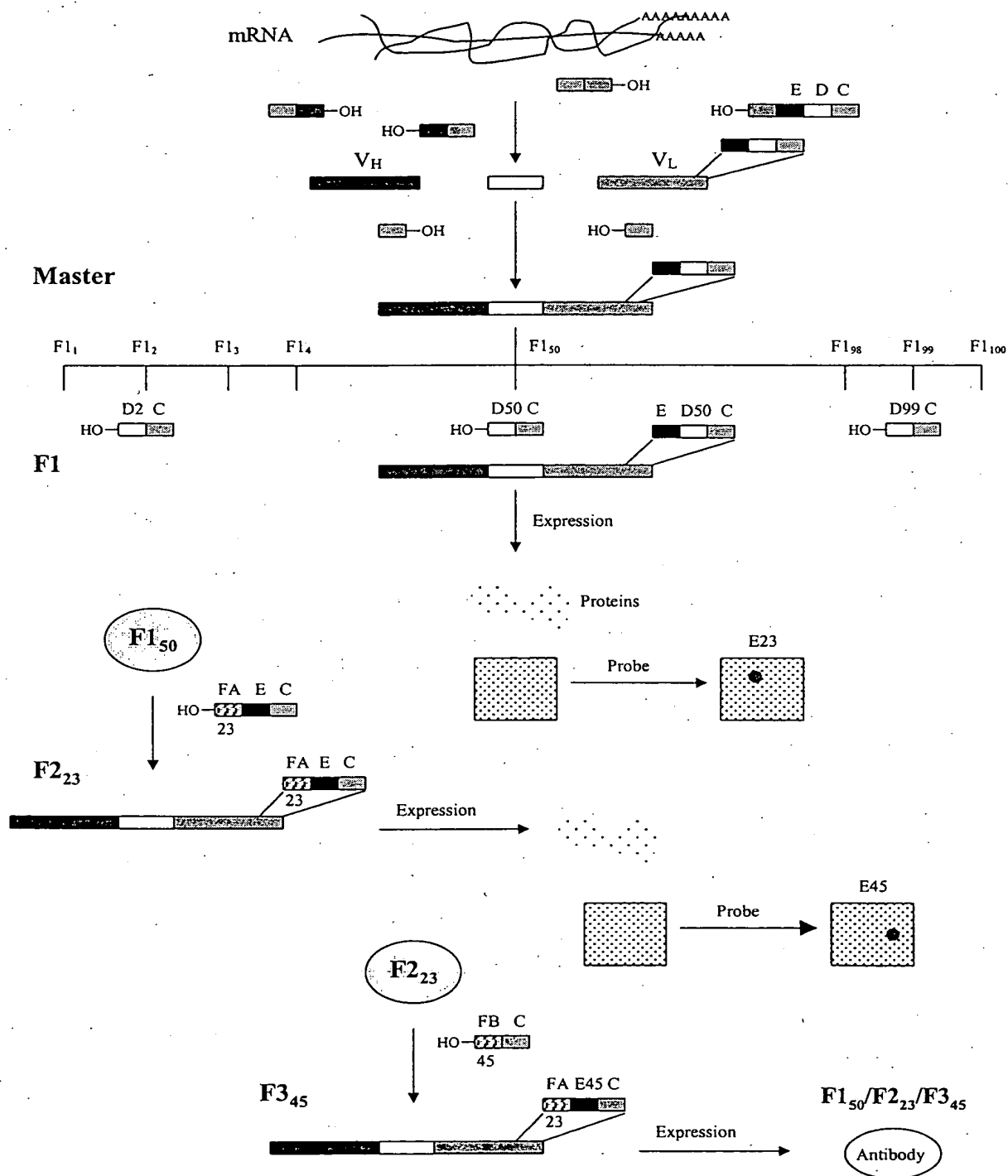
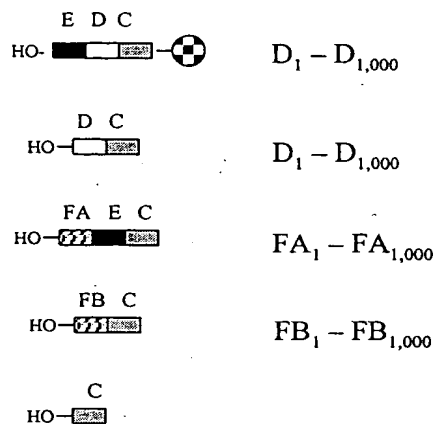


FIGURE 8

Physical elements to include in the kits and combinations

- *Anti-tag Arrays*[™]

- Primer sets



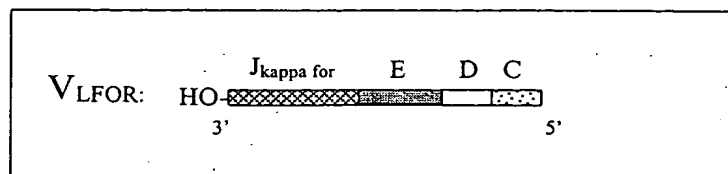
- Readers

- Software

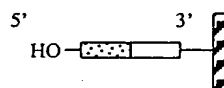
FIGURE 9

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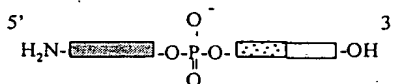
Making the V_LFOR primers: Solid phase synthesis



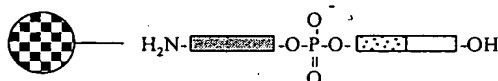
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extend by hybridizing with DNA patch and ligating

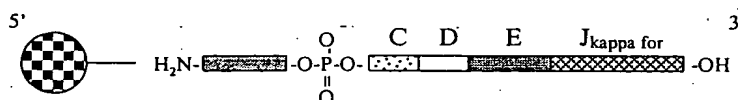
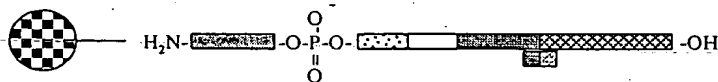
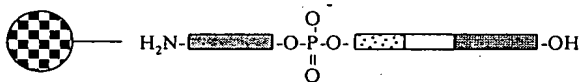
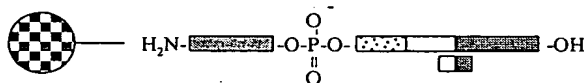
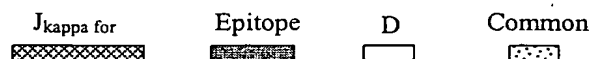
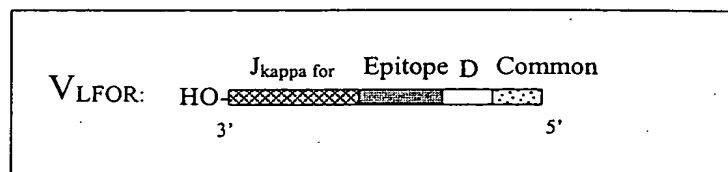
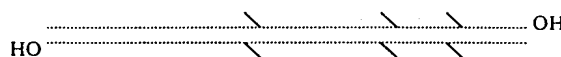


FIGURE 10

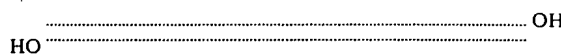
Making the V_{LFOR} primers: Overlapping hybridization



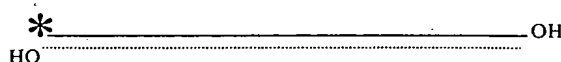
- Synthesize 4,028 different oligos:
 (26 for J_{kappa} for; 2,000 for Epitope; 2,000 for D; 2 for Common)
- 2. Assemble oligos for + and - strands of the different regions



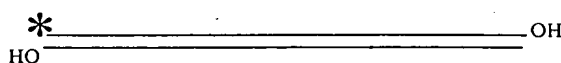
- 3. Ligase the assembled oligos



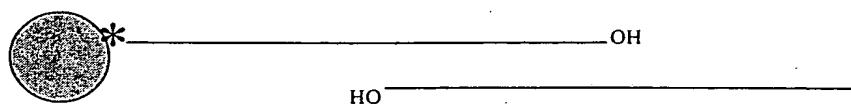
- 4. 1st strand synthesis with biotinylated primer



- 2nd strand synthesis with non-biotinylated primer



- 6. Bind to avidin coated magnetic beads and then denature



- 7. Purify non-biotinylated ssDNA

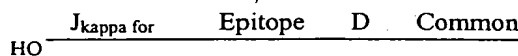


FIGURE 11

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Building the collection of antibody/tag pairs: Hybridoma screening

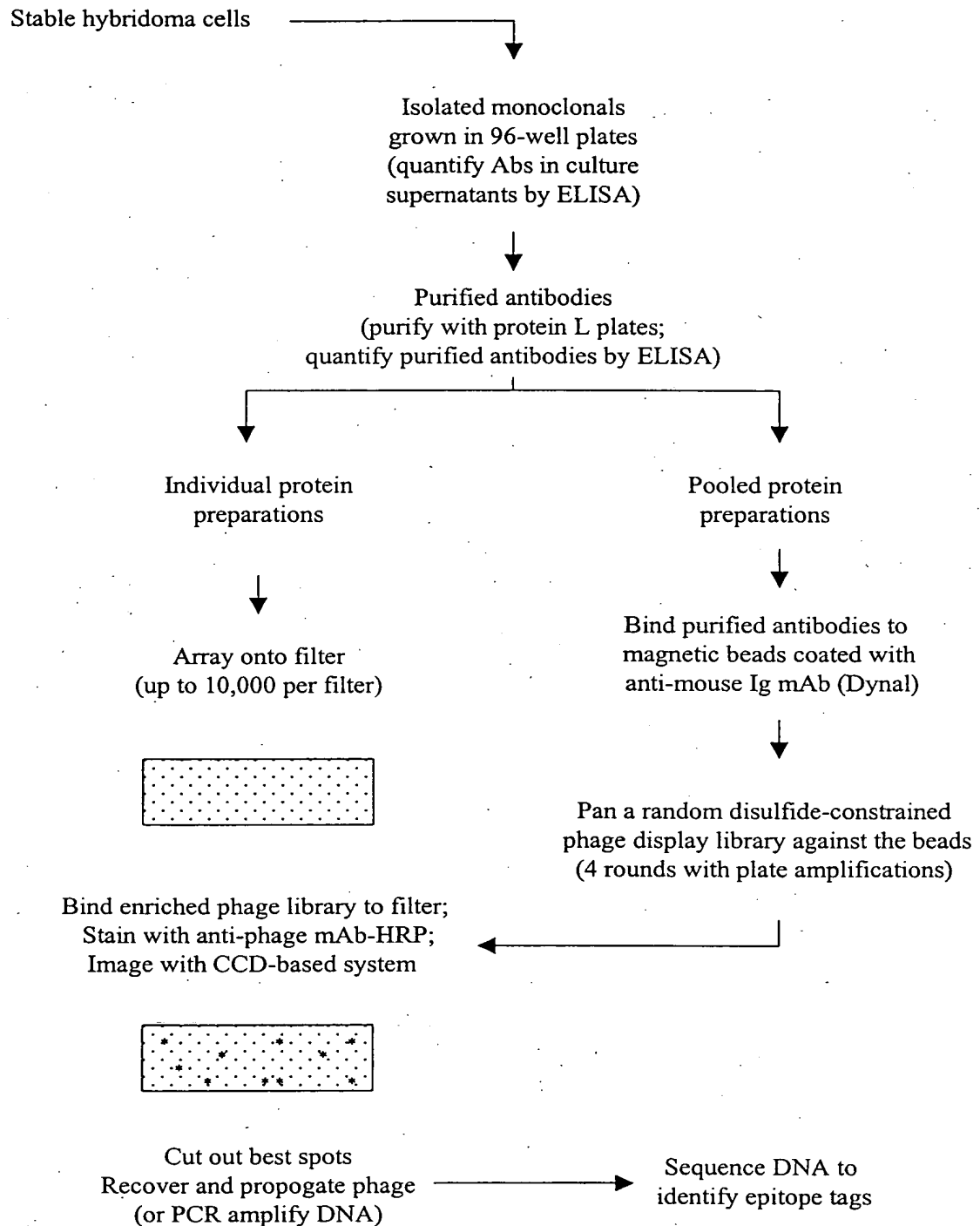


FIGURE 12

FIGURE 13A

TABLE 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)**I. V gene primary PCR***A. Human VH back primers (sense)*

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-CAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Human JH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT GCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuVk1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuVk2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuVk3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuVk4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuVk5aBACK	5'-GAA ACC ACA CTC ACG CAG TCT CC-3'
HuVk6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuVλ1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
HuVλ2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuVλ3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuVλ3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuVλ4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuVλ5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuVλ6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human J kappa forward primers (anti-sense)

HuJk1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJk2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJk3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJk4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJk5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human J lambda forward primers (anti-sense)

Hu Jλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
Hu Jλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
Hu Jλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

continues

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TABLE 3 Continued

FIGURE 13B

2. Linker fragment PCR

F. Reverse JH for scFv linker (sense)

—FR4 heavy— —linker—
 RHuJH1-2 5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH3 5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'
 RHuJH4-5 5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH6 5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'

F. Reverse Vk for scFv linker (anti-sense)

—FR1 light— —linker—
 RHuVk1aBACKFv 5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'
 RHuVk2aBACKFv 5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
 RHuVk3aBACKFv 5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
 RHuVk4aBACKFv 5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'
 RHuVk5aBACKFv 5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'
 RHuVk6aBACKFv 5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'

F. Reverse Vλ for scFv linker (anti-sense)

—FR1 light— —linker—
 RHuVλBACK1Fv 5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλBACK2Fv 5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλBACK3aFv 5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
 RHuVλBACK3bFv 5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'
 RHuVλBACK4Fv 5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλBACK5Fv 5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVλBACK6Fv 5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'

3. Pull-through primers for introduction of restriction sites*

G. Human VH back (Sfi) primers (sense)

HuVH1aBACKSfi —FR1 heavy—
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
 HuVH2aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'
 HuVH3aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG GAG TCT GG-3'
 HuVH4aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'
 HuVH5aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'
 HuVH6aBACKSfi
 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'

H. Human J kappa forward (Not) primers (anti-sense)

HuJk1FORNot —FR4 light—
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3'
 HuJk2FORNot
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACG TTT GAT CTC CAG CTT GGT CCC-3'

H. Human J kappa forward (Not) primers (anti-sense) (Continued)

HuJk3FORNot —FR4 light—
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3'
 HuJk4FORNot
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'
 HuJk5FORNot
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3'

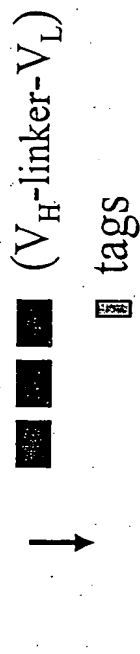
H. Human J lambda forward (Not) primers (anti-sense)

HuJl1FORNOT —FR4 light—
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3'
 HuJl2-3FORNOT
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3'
 HuJl4-5FORNOT
 5'-GAG TCA TTC TCG ACT TCG GCC CGC ACC TAA AAC GGT GAG CTG GGT CCC-3'

*Recognition site for restriction enzyme is underlined.

step I

Tag and assemble immunoglobulin genes



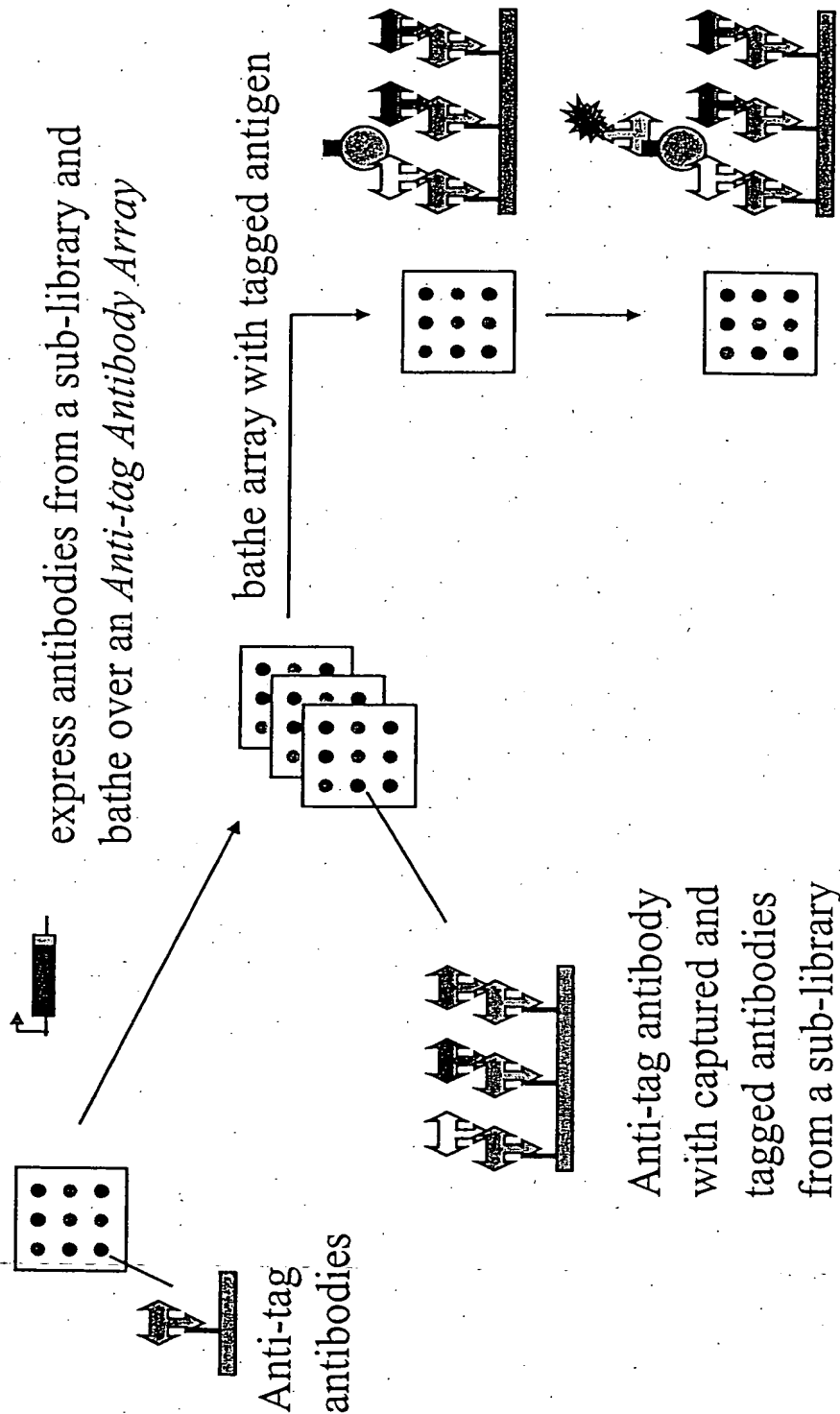
Create 1,000 sub-libraries by separate PCR amplification reactions using tag-specific PCR primers



1,000 sub-libraries

FIGURE 14A

step II



ID spot containing the antigen
with a labeled developing Ab

FIGURE 14B

step III

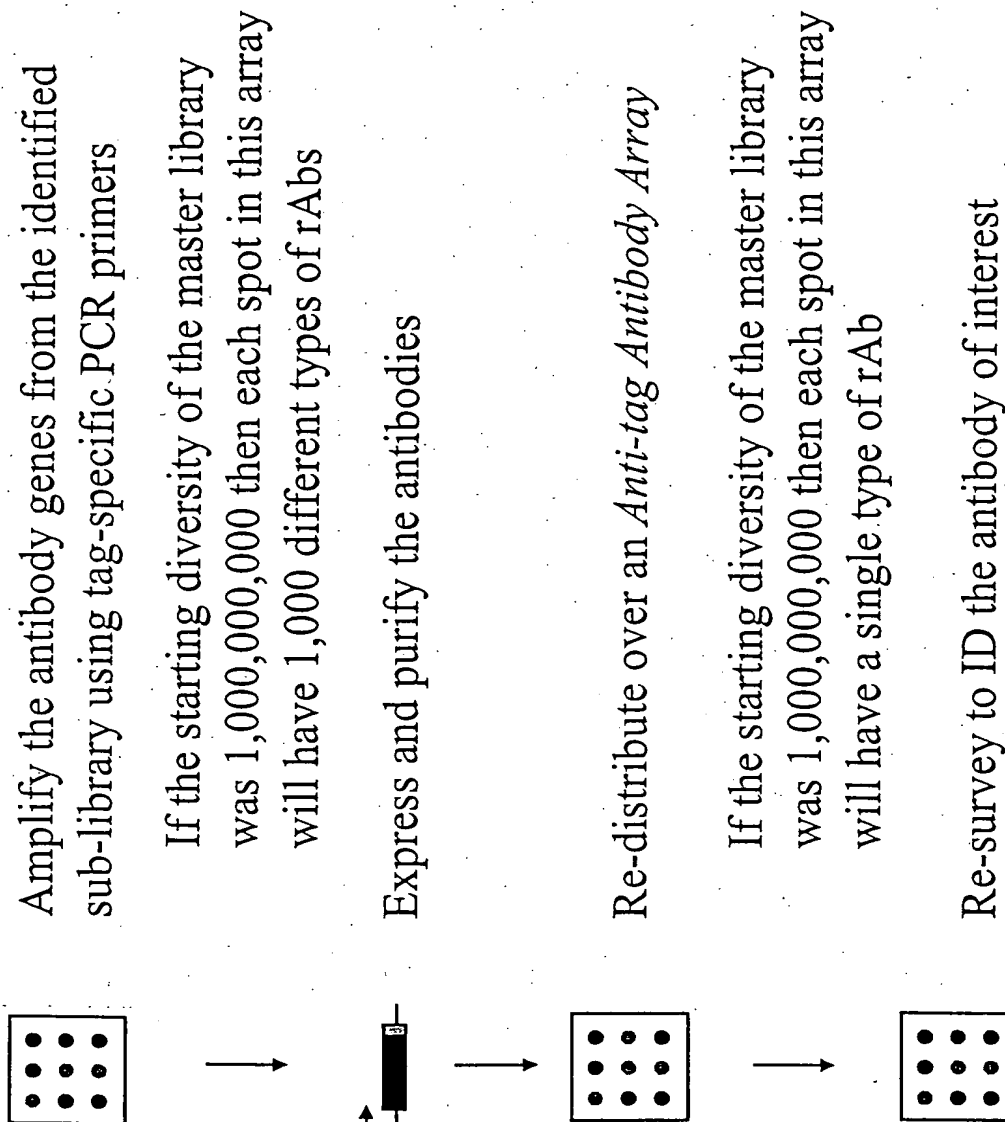
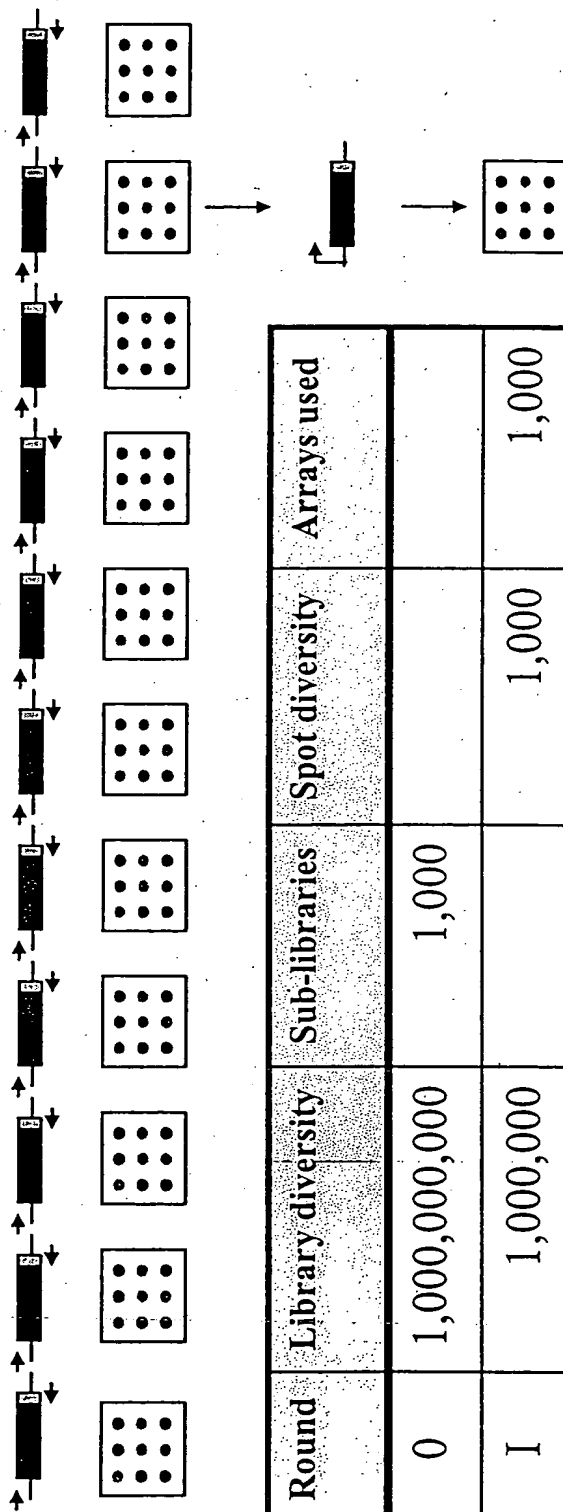


FIGURE 14C

summary

master library

1,000 sub-libraries



Round	Library diversity	Sub-libraries	Spot diversity	Arrays used
0	1,000,000,000	1,000		
I	1,000,000		1,000	1,000
II	1,000		1	1

FIGURE 14D

- Modification searches

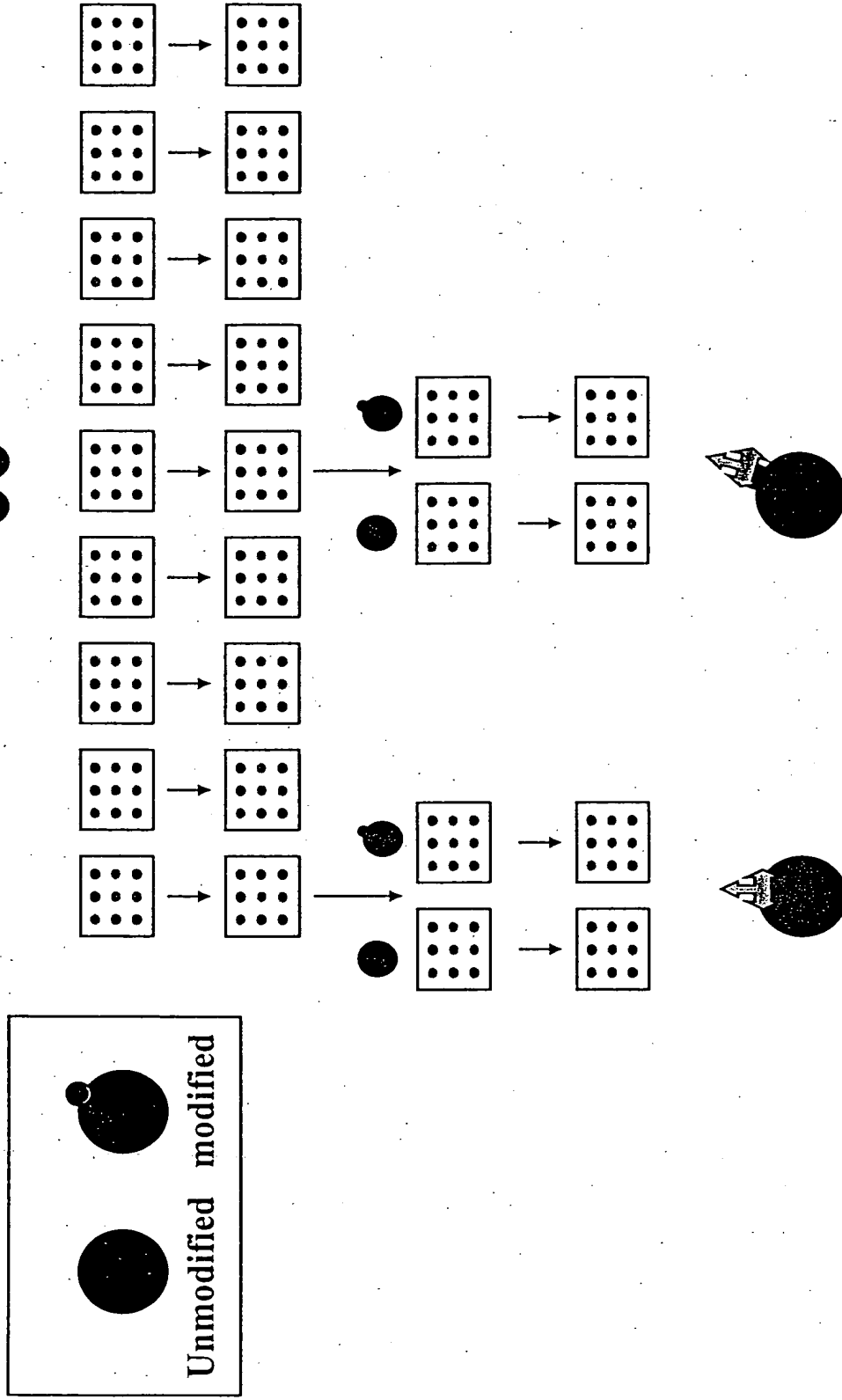


FIGURE 15

Round Arrays Bait Probe

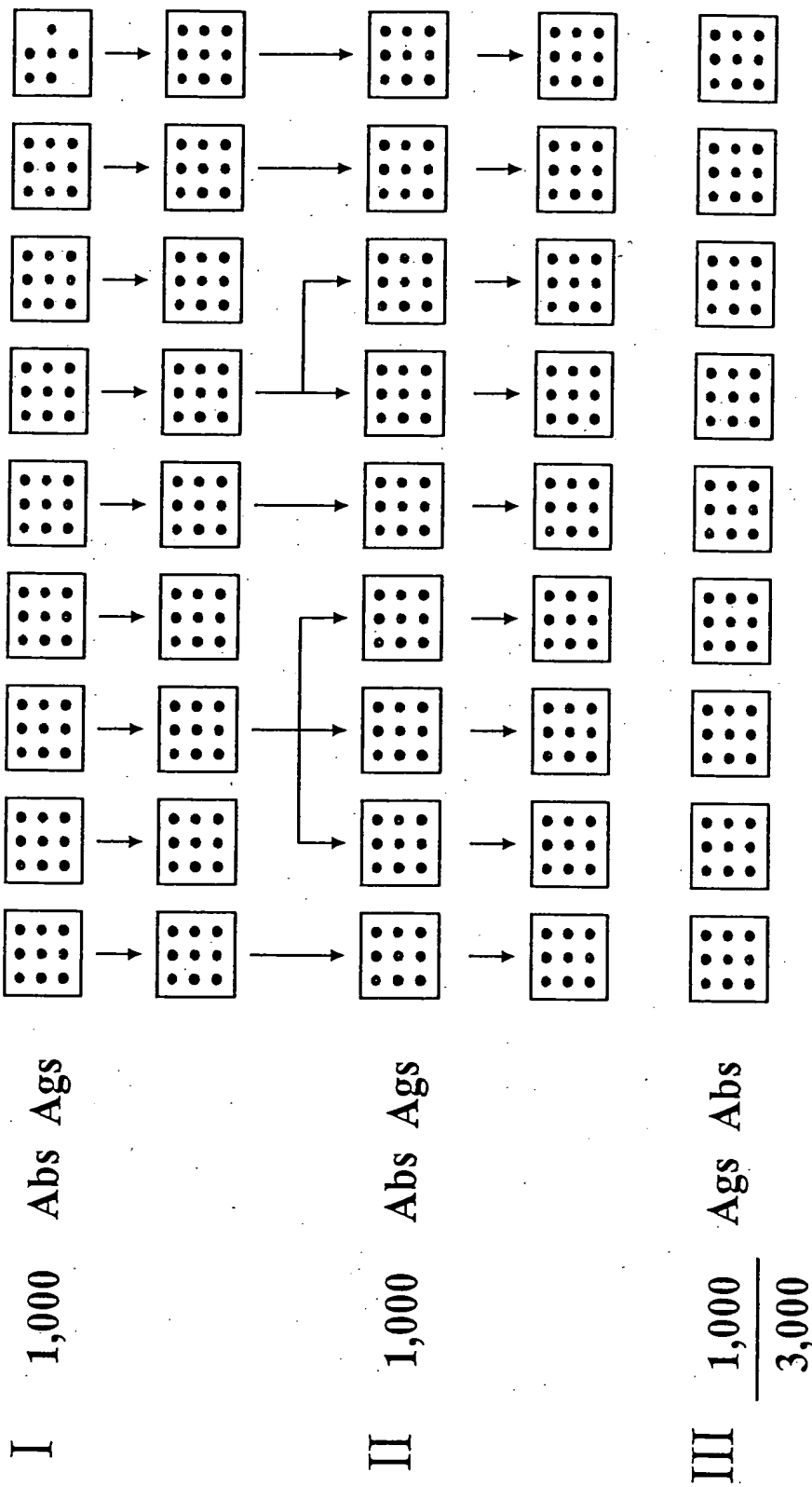
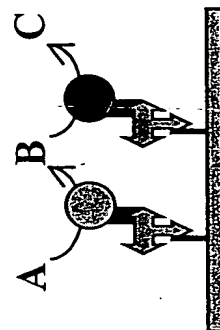
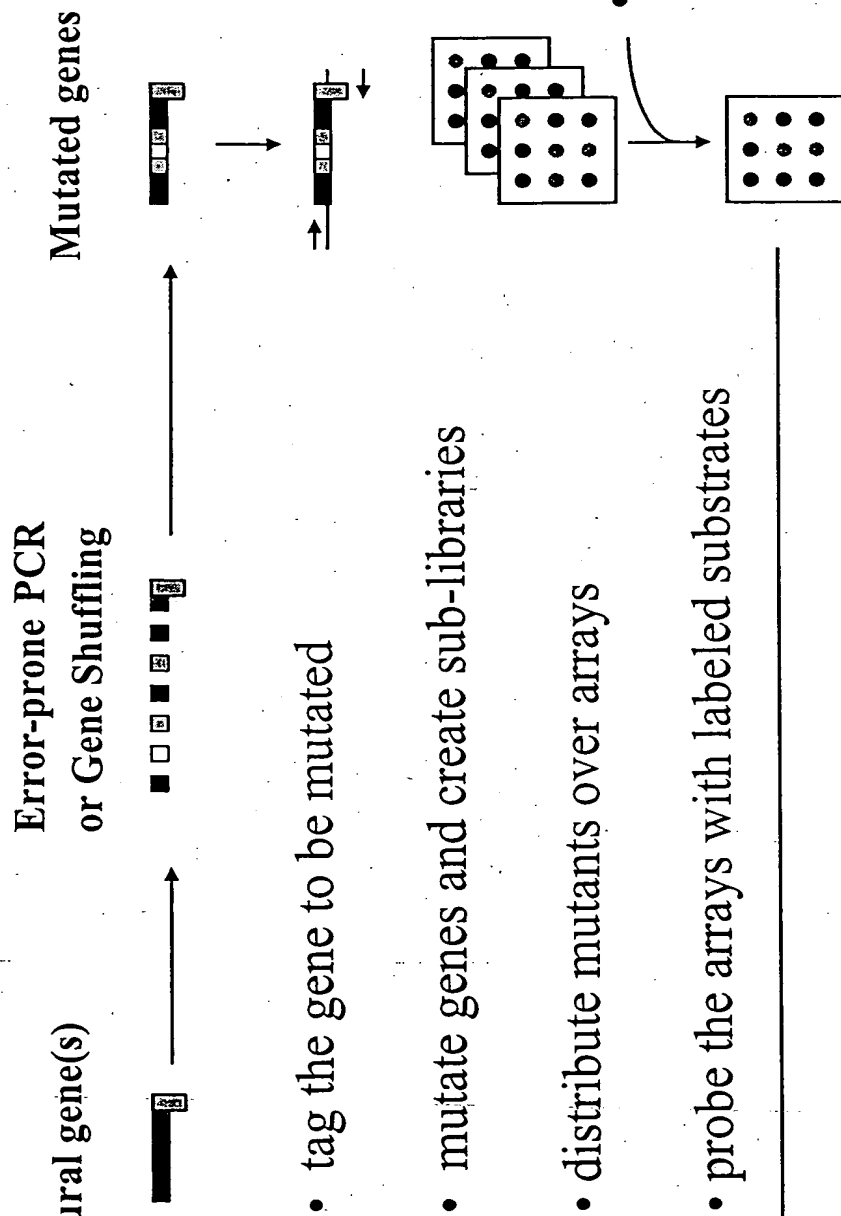


FIGURE 16

Enzyme engineering



Spots can contain mixtures of enzymes for detection or pathway engineering

FIGURE 17

Protein interaction mapping

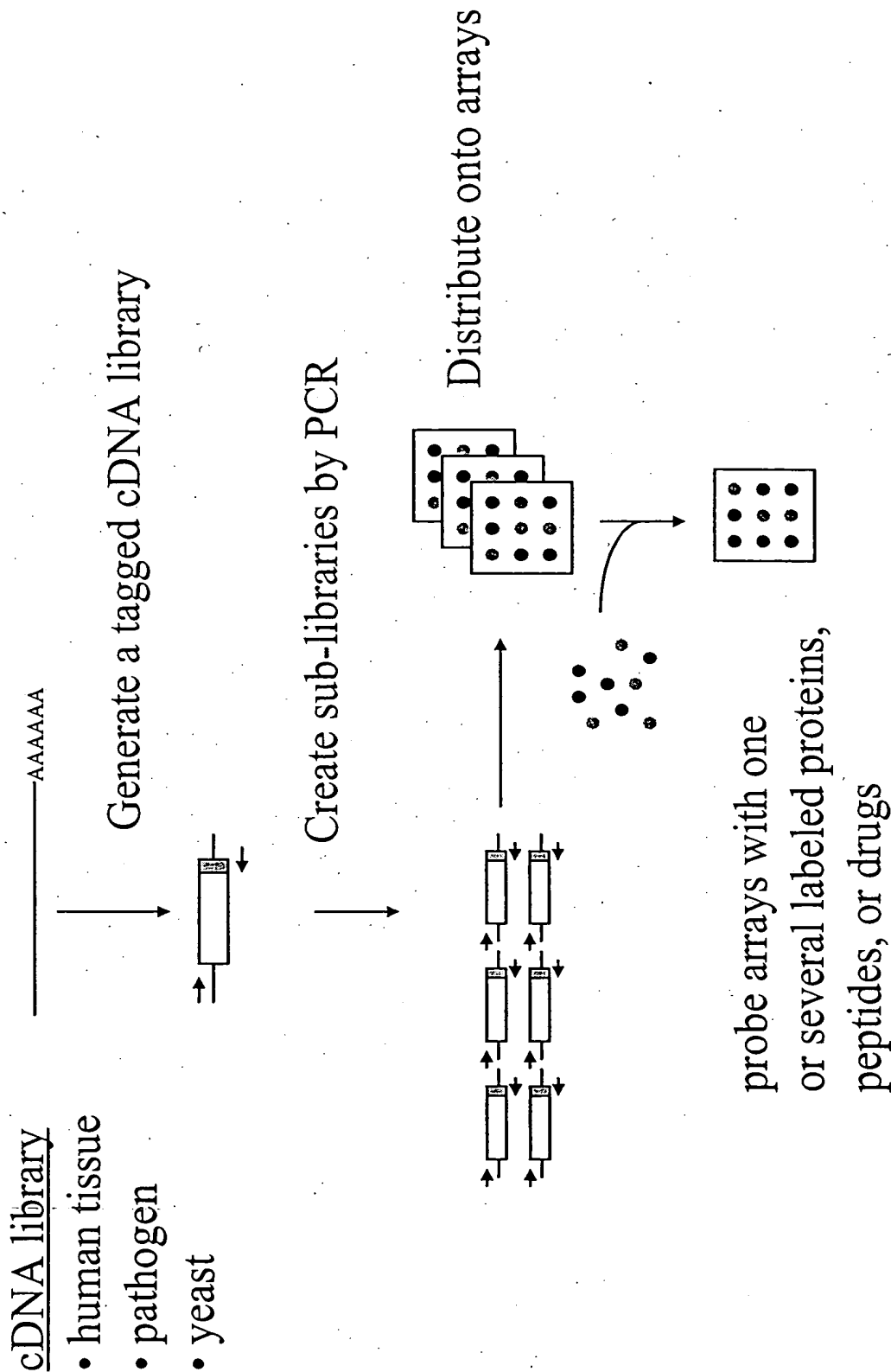
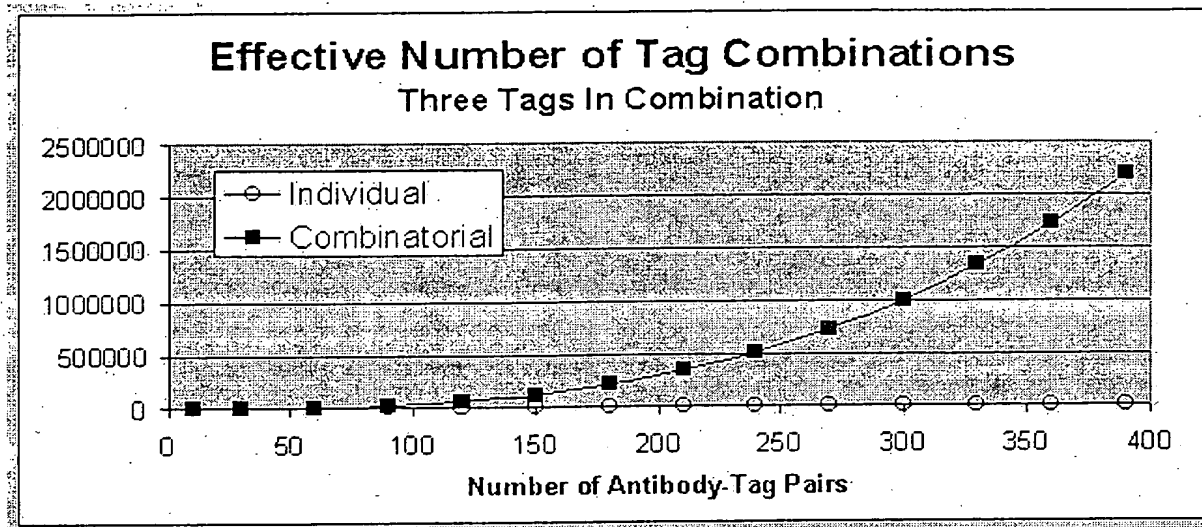


FIGURE 18

FIGURE 19



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